Computational studies with Penicillin G

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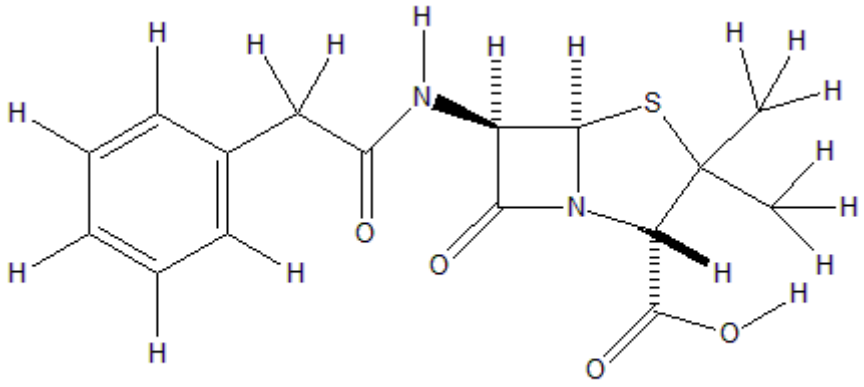
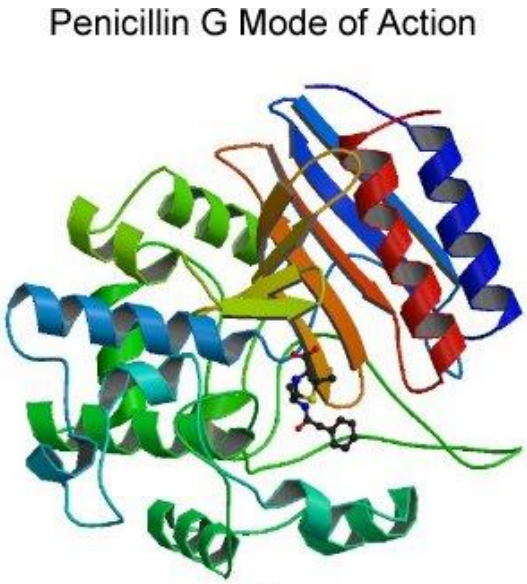


1

Fig. 1

The β-lactam antibiotics, such as penicillin G (benzylpenicillin, PenG) (**1**, Fig. 1), itself discovered in 1929, have found global widespread use in a number of bacterial infections, including pneumonia, strep throat, syphilis, necrotizing enterocolitis, diphtheria, gas gangrene, leptospirosis, cellulitis, and tetanus [1]. It is often administered intravenously as a water-soluble sodium salt. Penicillin G kills mainly Gram-positive bacteria by inhibition of DD-peptidases and DD-transpeptidases.

Their main mode of action is irreversible binding to these enzymes. However, there is increasing global concern in widespread use due to evolutionary bacterial resistance to penicillin [2]. Some strains of *S. enterica* are resistant to penicillin G. Mutational alterations in (penicillin-binding proteins) PBPs can confer resistance either by reducing binding of the antibiotic to the active site or by evolving a β-lactamase activity that degrades the antibiotic [2].



Figs. 2 & 3. Penicillin G forms an irreversible complex with Streptomyces R61 DD-peptidase.

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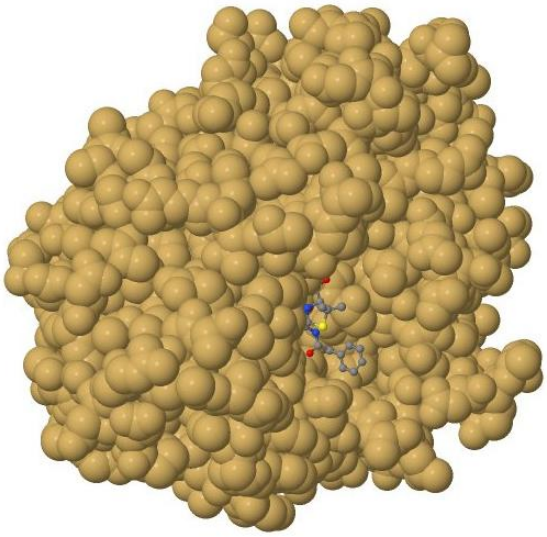


Fig. 3

The structure the enzyme-penicillin complex and active site is shown (Figs. 2 & 3): the penicilloyl acyl enzyme complex of the Streptomyces R61 DD-peptidase with penicillin G [3]. The atomic arrangement of the individual amino acids in the polypeptide chains are shown collectively in a light brown colour from the crystal structure.

Computational Studies

A semi-empirical study of penicillin G was undertaken a few years ago [4]. It was of interest to use the MOPAC semi-empirical PM7 method [5, 6] to determine its equilibrium geometry (an energy minimum on the PES) or as an anion (water solvation model) and compare with other more costly quantum chemistry methods such as *ab initio* or DFT using Firefly [7] or GAMESS [8] and to study other electronic 3D properties with Jmol [9]. Avogadro [10] or Jmol were used for the 3D visualisaions of application program output data. The optional input geometry optimisation correction factor for a -CO-NH-peptide linkage [4] (MMOK with MOPAC) was not necessary for these molecules using MOPAC 2016 (an example without MMOK is also provided: PenG1no.txt, PenG1no.mgf).

Some of the figures and data from the quantum chemistry molecular modelling studies in this article have been recently published by the same author on Twitter [11].

First of all, the PM7 of penicillin **1** is shown (Fig. 4). It was surprising to see a compact structure after PM7 geometry optimisation (after an initial MMFF94 optimisation using Avogadro) in gas phase (PenG1.txt, PenG1.pdb, PenG1.mgf, PenG1freq.txt), as the structure was expected to be more spread out (as seen after initial MM studies). Heat of formation (ΔH_f) = -130.88 Kcal/mol; -547.60 KJ/mol.

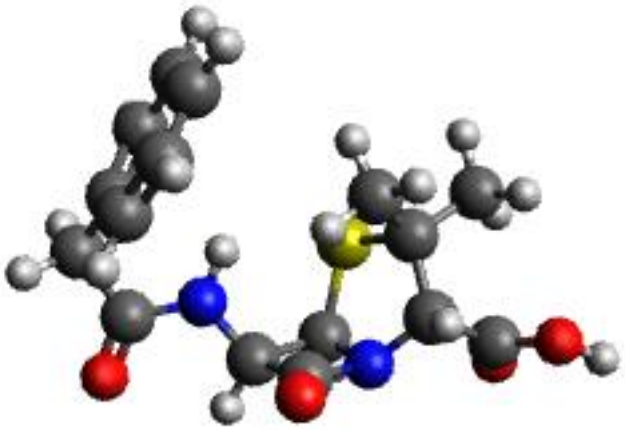
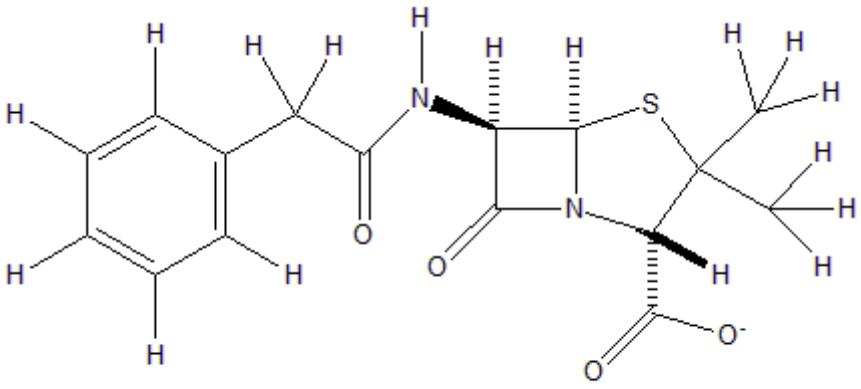


Fig. 4



2

Fig. 5

Next, and to better simulate aqueous conditions, the anion **2** (Fig. 5)was subjected to a similar PM7 geometry optimisation (after initial MMFF94 optimisation) using the water solvation model with MOPAC (PenG2.txt, PenG2.pdb, PenG2.mgf, PenG2freq.txt). The expected 3D geometry is shown (Fig. 6) and Avogadro atom labelling is arbitrary (Fig. 7). For example, the C11 C9 N1 C8 torsion angle = 120.44 degrees. Frequency calculations on molecule **1** only revealed positive frequencies (no negative/imaginary frequencies) and therefore a true energy minimum at this level of theory. The anion **2**, however, revealed several imaginary frequencies.

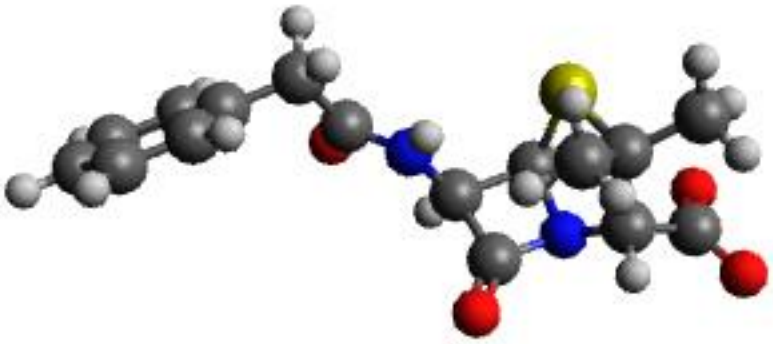


Fig. 6

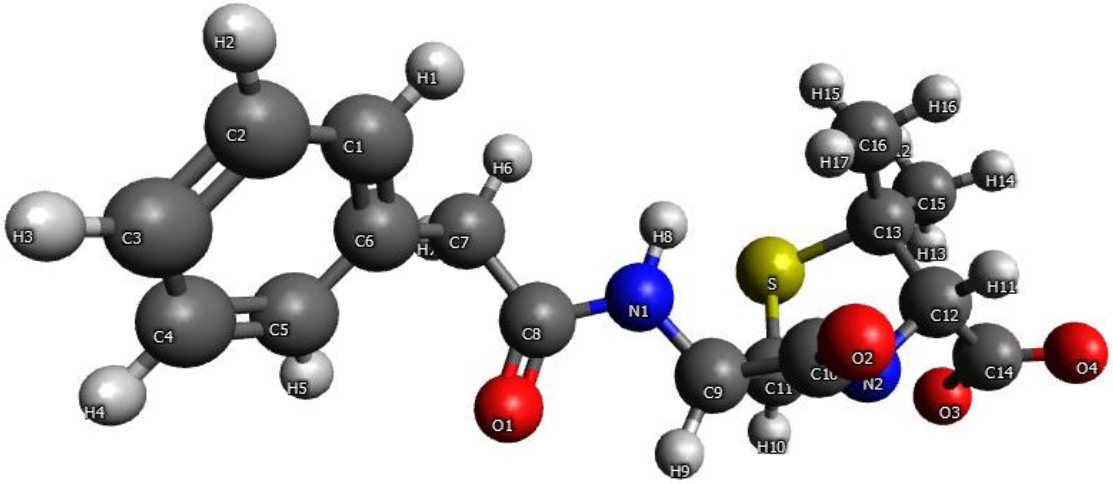


Fig. 7

The electrostatic potential is shown (Jmol) (Fig. 8):

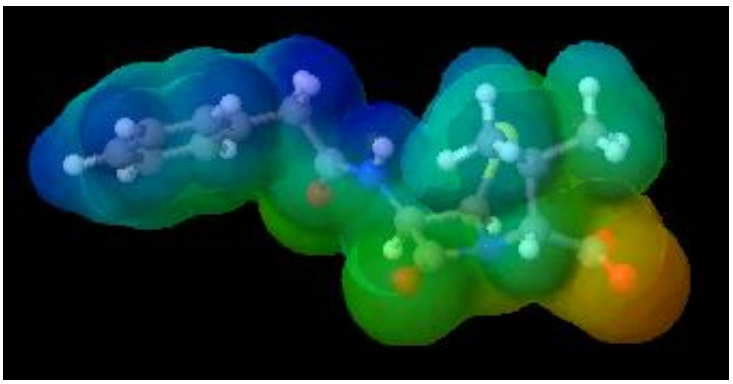


Fig. 8

As well as the Van der Waals dot surfaces (Jmol) (Fig. 9):

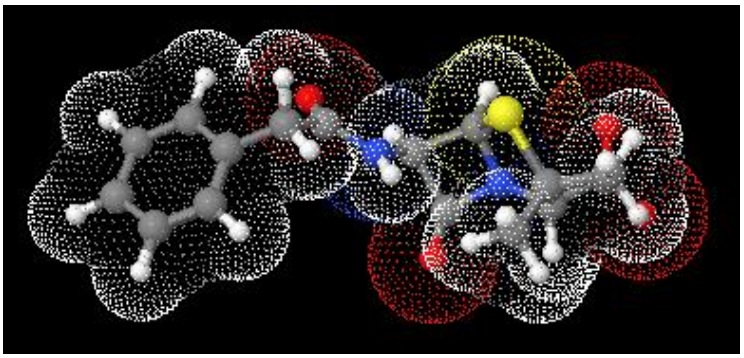


Fig. 9

After a DFT (RB3LYP/6-31G(d)) geometry optimisation, an expected similar structure is seen (PenG3.txt, PenG3.pdb) (Fig. 10 and 11). The traditionally popular 6-31G(d) basis set is a good compromise between computational cost and accuracy. PenG3.txt is final output after a number of runs (the original starting geometry is therefore not included there via MMFF94). Energy = -1429.14183 Hartrees. However, the frequency analysis (PenG3freq.txt) found one imaginary (negative frequency) with this geometry so it is not a true energy minimum, but a saddle point on the PES and therefore a transitional structure requiring more calculations to reach a true energy minimum! The geometries here are for comparative purposes only.

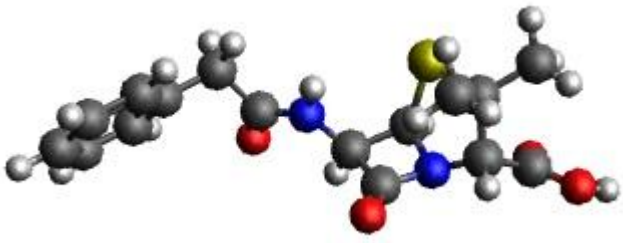


Fig. 10 Result from the DFT studies

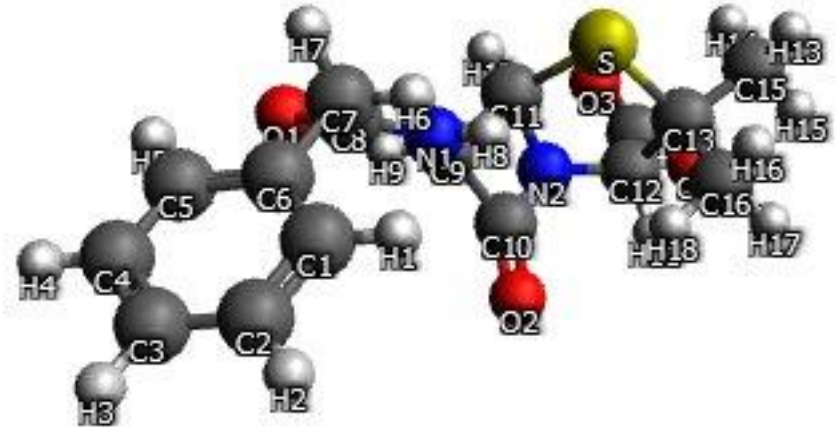


Fig. 11

Example torsion angles for **1** = C12 N2 C11 S: -29.92; C11 C9 N1 C8: 122.96 degrees. Example bond lengths for Penicillin G from DFT, e.g. C1-C2: 1.4; N1-H9: 1.01; C11-S: 1.85; C8-N1: 1.38; O4-H12: 0.98; O1-C8: 1.22Å.

The DFT output (PenG3.pdb) was then subjected to further geometry-optimisation calculations using the *ab initio* (Hartree-Fock) functional method at the 6-31G(d) level of theory [8] using GAMESS. This time an energy minimum similar in nature (Figs. 12 & 13) to the PM7 geometry was obtained with no imaginary frequencies (PenG4freq.txt, PenG4.pdb). Total energy = -1422.26262 Hartrees. Post RHF MP2 energy data [7] is also provided for this geometry (MP2.txt) for comparative purposes.

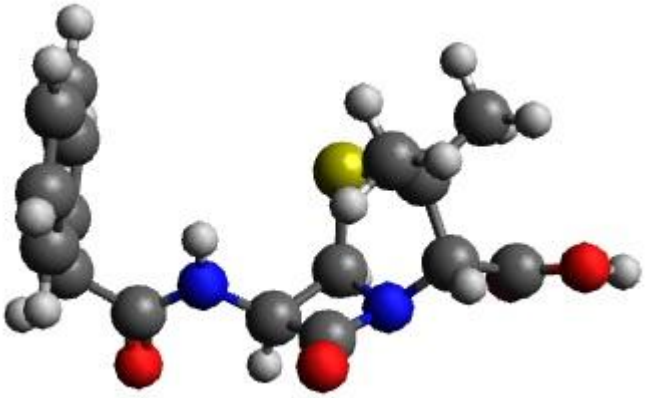


Fig. 12. RHF/6-31G(d) geometry of **1.**

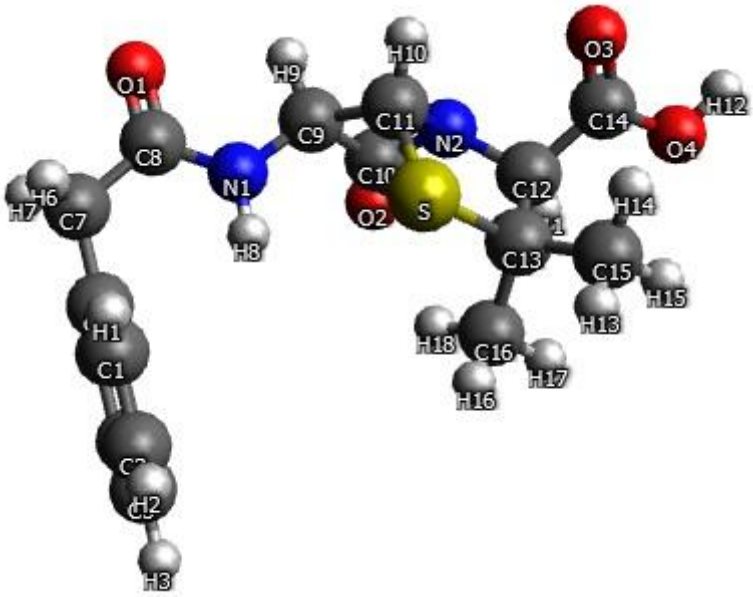


Fig. 13.

Example torsion angles for **1** = C12 N2 C11 S: -28.12; C8 N1 C9 C11: 130.71 degrees. Example bond lengths for Penicillin G from HF, e.g. C1-C2: 1.39; N1-H8: 0.99; C11-S: 1.83; C8-N1: 1.36; O4-H12: 0.95; O1-C8: 1.20Å.

When the molecule (presumably as a solvated anion) enters the enzyme to form an irreversible complex and inhibit its activity for bacteria cell wall biosynthesis, the molecule is expected to adopt a different conformation due to the formation of intermolecular bonds (and including hydrogen bonds) and mimimisation of steric repulsion. It is, however, seen in the crystal state (Figs. 2 & 3).

NBO and ELF studies

It was of interest to investigate further electronic properties, namely *natural bond orders* (NBO) [12] and the *electron localisation function* (ELF) [13] of these molecules using various computer programs. Selected NBO output [12] (PenG1NBO.txt, PenG2NBO.txt, PenG3NBO.txt and PenG4NBO.txt) is available as several files (PenG1NBO.31, PenG1NBO.37; PenG2NBO.31, PenG2NBO.37; PenG3NBO.31, PenG3NBO.37; PenG4NBO.31 and PenG4NBO.37), as well as selected ELF output [13] (PenG1ELF.pdb, PenG1_attractors.txt; PenG2ELF.pdb, PenG2_attractors.txt ; PenG3ELF.pdb, PenG3_attractors.txt; PenG4ELF.pdb and PenG4_attractors.txt). A high quality grid option was used with Multiwfn when generating attractors (and basins). The attractors (and some of their labels) for PM7 geometry-optimised structure **1** (PenG1), its anion **2** (PenG2) and again for **1** from the DFT structure (PenG3) and RHF (PenG4); the attractors are seen in a light green colour (Figs. 14 - 21):

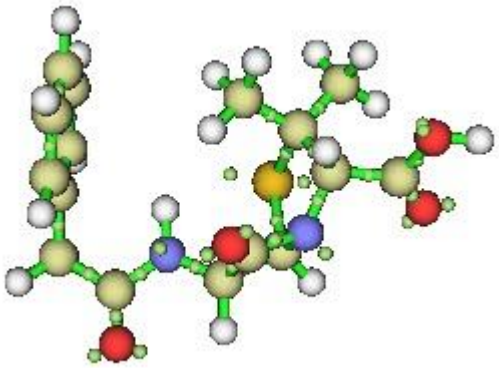


Fig 14. ELF attractors for PM7 geometry; PenG1.

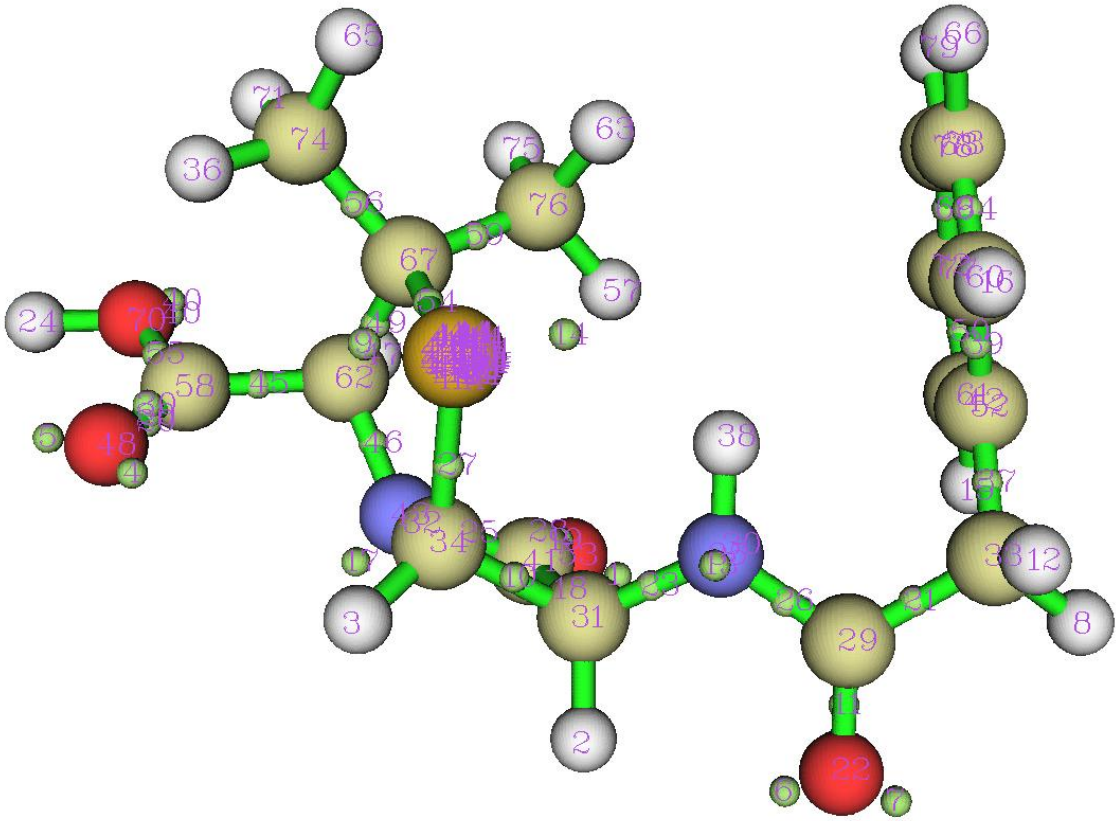


Fig. 15. PenG1.

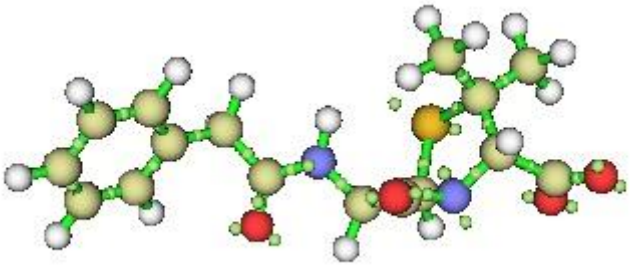


Fig. 16. ELF attractors for anion **2** (PM7 geometry); PenG2.

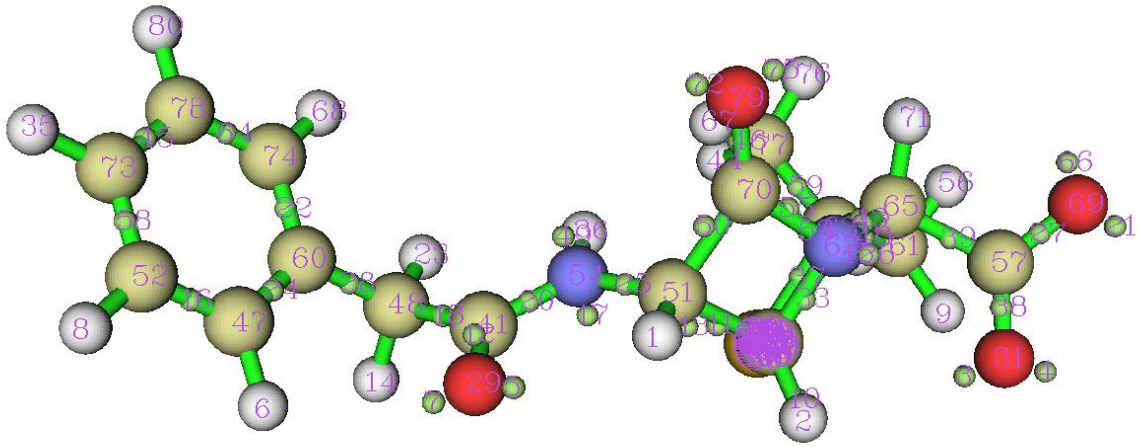


Fig. 17. PenG2.

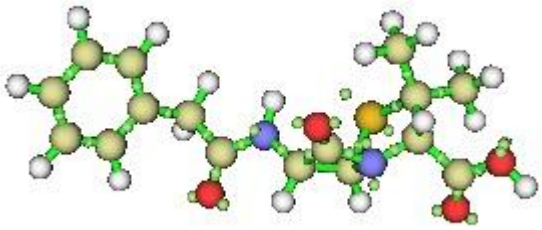


Fig. 18. ELF attractors for the DFT studies; PenG3.

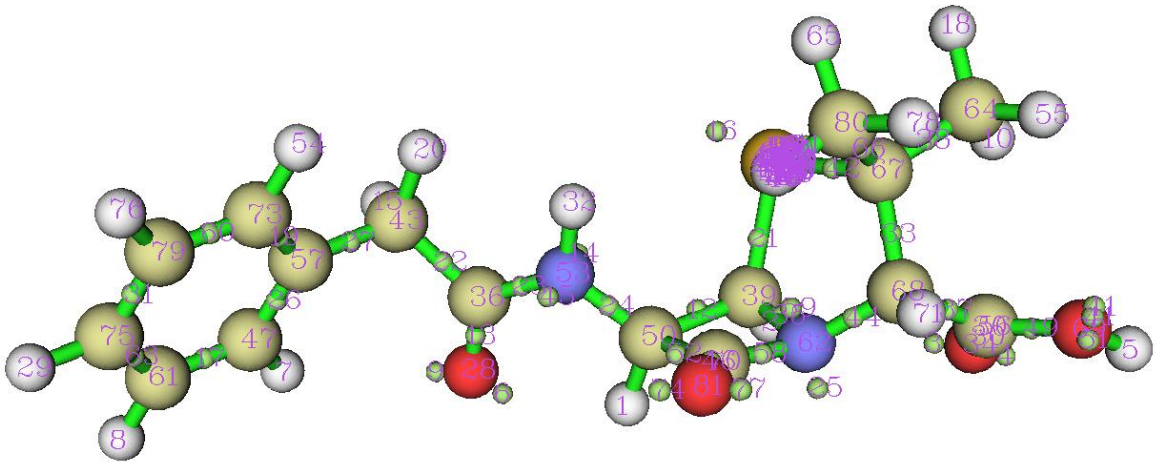


Fig. 19. PenG3.

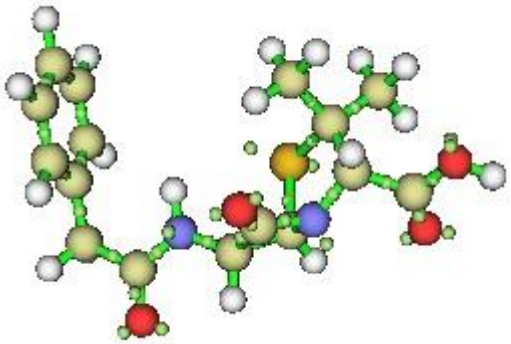


Fig. 20. ELF attractors for the *ab initio* studies; PenG4.

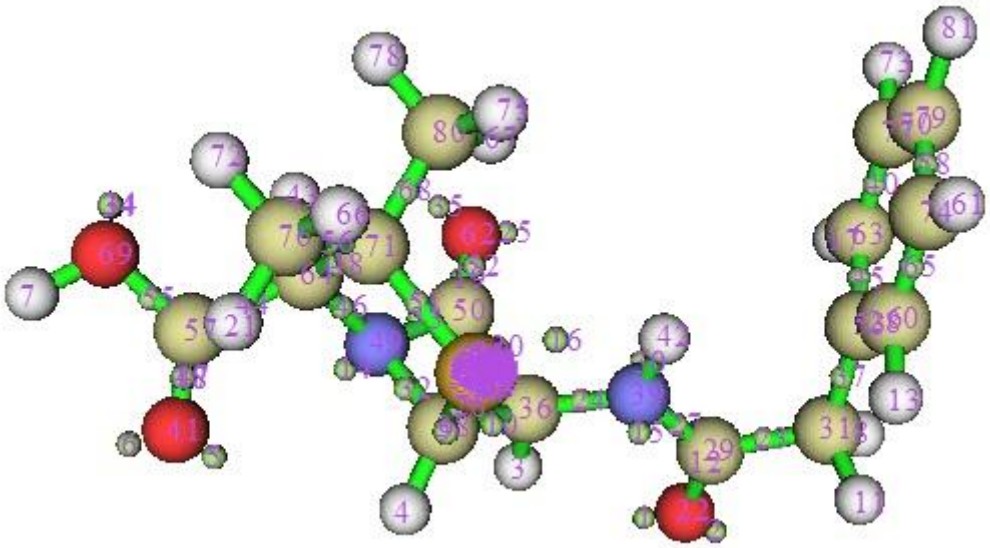


Fig. 21.

The attractors can also be visualised via Avogadro (Fig. 22), as shown below (e.g. PenG3ELF.pdb); notice the increase size around the sulphur atom due to the extra attractors:

Fig. 22. Avogadro attractor visualisation.

The Savin ELF surfaces [14] (isovalue 0.8) are shown in blue via the Firefly energy output files (PenG1NBO.txt, PenG2NBO.txt , PenG3NBO.txt and PenG4NBO.txt) using Gabedit 2.4.8 [15] (Figs. 23-26):

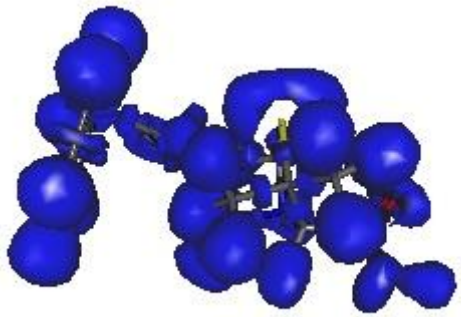


Fig. 23. Savin ELF surfaces; PenG1.

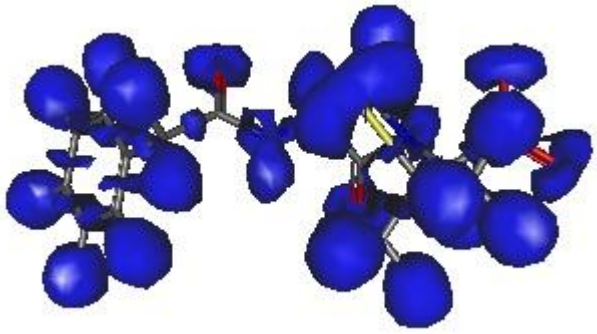


Fig. 24. PenG2.

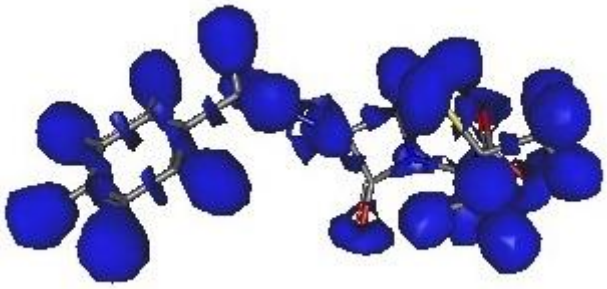


Fig. 25. PenG3.

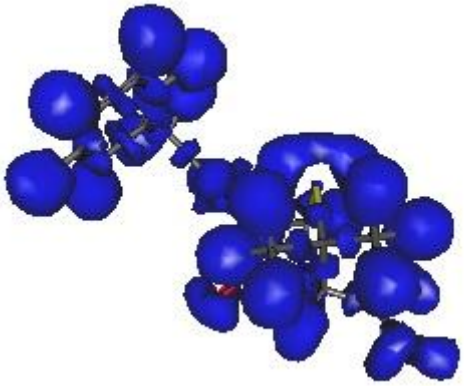


Fig. 26. PenG4.

Conclusion

The geometry-optimised structures for penicillin G have been determined from semi-empirical PM7 calculations after an initial molecular mechanics optimisation. The results from the DFT (B3LYP 6-31G(d)) calculations are yet to lead to a successful true energy minimum on the PES from its frequency analysis (its saddle point was found), but probably a geometry close to it. Data is available for further investigation. Although penicillin G (attempted DFT geometry) and its anion (PM7 geometry, H2O solvation model) revealed similar structures, the geometry for Penicillin G from the PM7 study and *ab initio* (HF/6-31G(d)) study was more compact for an energy minimum on the PES, its likely global minimum. ELF surfaces and attractors were observed as expected.

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Abstract

A study has been undertaken to investigate the structure of Penicillin G (benzylpenicillin) and its anion by quantum chemistry molecular modelling studies. The geometry-optimised structures have been determined from semi-empirical PM7 calculations (presumed global minima after an initial molecular mechanics geometry optimisation). Although an equilibrium geometry was found from DFT studies (B3LYP 6-31G(d)), the frequency analysis suggested is not a true energy minimum, requiring further calculations. Program out data is provided (e.g. bond lengths and torsion angles). Even though penicillin G (a DFT geometry) and its anion (PM7 geometry, H2O solvation model) revealed similar structures, the geometry for Penicillin G from the PM7 and ab initio (HF/6-31G(d)) study was more compact for its likely global minimum on the PES (with no imaginary frequencies). These molecules were evaluated further using NBO and ELF analyses revealing the expected ELF surfaces and attractors.

Keywords: Penicillin G, benzylpenicillin, PenG, molecular modelling, semi-empirical, PM7, DFT, B3LYP 6-31G(d), anion, water, solvent, enzyme, inhibition, DD-peptidases, bacteria resistance, antibiotic, biochemistry, NBO, ELF.